BIOLOGICAL CONTROL OF METHANE IN COAL MINES, COAL STORAGE SILOS AND SHIP HOLDS

PHASE VII

A REPORT BY

GEMINI BIOCHEMICAL RESEARCH LTD.

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SUMMARY

An active culture was rejuvenated from a sample retained from the second large scale test. This new culture was grown in the 30 litre bioreactor and was partially characterized.

The new culture was not as active as the original strain and, in addition, was not as stable on storage.

This new strain was used for a number of small scale tests, both in Calgary and in Sydney, Nova Scotia.

Two small scale tests carried out using porous coal gave very encouraging results. Greater than 90% of the methane was oxidized and good penetration of oxygen into the coal pile was observed.

A larger test carried out in Sydney was not as successful. This may have been due to the instability of the bacterial cells. In addition, the method used to measure methane oxidation may have underestimated the percentage of methane oxidized.

Tests with hydrogen peroxide were not successful since the bacterial activity was inhibited at low concentrations of the hydrogen peroxide.

The use of long lasting liquid foams is an area which merits further study. When the bacteria were immobilized in a protein foam they were completely active. However, a chemical foam inhibited the bacteria.

Small scale experiments in which the cells were immobilized onto glass fibres appeared to be successful. However, this experiment was stopped before confirmatory data were obtained.

RÉSUMÉ

On a rajeuni une culture active à partir d'un échantillon conservé du deuxième essai à grande échelle. Cette nouvelle culture avait crû dans le bioréacteur de 30 litres et avait été caractérisée partiellement.

Le nouvelle culture n'était pas aussi active que la race originale et, en outre, elle n'était pas aussi stable pour la conservation.

Cette nouvelle race a servi à un certain nombre d'essais à petite échelle, à Calgary et à Sydney (Nouvelle-Écosse).

Deux essais à petite échelle exécutés à l'aide de charbon poreux ont donné des résultats très encourageants. Plus de 90 % du méthane a été oxydé et on a observé une bonne pénétration de l'oxygène dans le stock de charbon.

Un essai plus important a été exécuté à Sydney, mais il n'a remporté autant de succès. Cela peut être imputé à l'instabilité des cellules bactériennes. De plus, la méthode employée pour mesurer l'oxydation du méthane peut avoir sous-estimé le pourcentage de méthane oxydé.

Des essais avec du peroxyde d'hydrogène n'ont pas réussi car l'activité bactérienne était inhibée aux faibles concentrations du peroxyde d'hydrogène.

L'emploi de mousses liquides de longue durée est un domaine qui mérite une étude plus poussée. Lorsque les bactéries étaient immobilisées dans une mousse aux protéines, elles étaient tout à fait actives. Cependant, une mousse chimique a inhibé les bactéries.

Les expériences à petite échelle selon lesquelles les cellules étaient immobilisées sur des fibres de verre ont semblé réussir. Cependant, l'expérience a été interrompue avant d'obtenir des données confirmatives.

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INTRODUCTION

For the past several years Gemini Biochemical Research Ltd. has been researching the use of methane oxidizing micro-organisms to control methane in coal mines, coal storage silos and ship holds. Several phases of the work have been completed.

Phase I included a literature review on the properties of methane oxidizing bacteria to identify if conditions in coal mines and coal storage vessels would be suitable environments for these organisms. In addition, a review of the latest research from Russia, Eastern Europe and India was included (1).

The literature review showed that the methane oxidizing bacteria had physiological properties which are suitable for use in coal mining environments. In particular, their pH profiles, temperature optima and nutritional requirements were found to be appropriate.

The survey of the recent field tests in various countries showed that most of the tests yielded positive results when used under actual mining conditions but that there was a paucity of information on the microbiological characteristics of the organisms used and that not enough data were presented to critically assess the validity of the results.

Based on the results presented in the report from Phase I (1), Phase II was initiated and the results of this phase of the project are summarized below (2).

Water and coal samples were obtained from five mines in Western Canada and two from Cape Breton. Chemical analysis of the water samples and coal leachates from the Western Canadian mines showed that conditions in these mines were appropriate for the growth of methane oxidizing bacteria. In

particular, the pH of the mine waters was in the correct range (pH 7.0-8.5) and there did not appear to be any toxic compounds present in the water. The pH levels in water samples obtained from the Cape Breton mines were found to be higher than expected, most samples being in the range 7.0-8.5. A few samples with low pH probably reflect the activities of sulphur oxidizing bacteria on the pyritic sulphur in the coal.

Methane oxidizing bacteria were found to be present in all water samples examined from Western Canadian mines.

One strain of methane oxidizing organism was grown in larger quantities and its growth requirements were partially characterized. The organism grew in a simple inorganic (Bushnell Haas) medium when incubated in a methane/air (20/80; v/v) mixture. The optimal pH (8.0) of this organism was very close to the average pH levels found in the Western Canadian mine waters.

The organisms retained 60% of their initial activity after a period of 100 days in contact with a number of low sulphur Western Canadian coal samples. However, they rapidly lost activity when in contact with an unwashed, high sulphur Cape Breton coal. This loss in activity is believed to be due to the oxidation of pyritic sulphur, present in the coal, to sulphuric acid thus lowering the pH of the liquid phase to below that at which the methane oxidizing bacteria retain maximum activity.

When the methane oxidizing bacteria were added to partially degassed Western Canadian coals, the organisms oxidized the methane as it desorbed from the coal. No methane was detectable after seven days whereas tests with no added bacteria showed a 7% methane concentration.

Experiments with a dynamic test set up showed that the

organisms were able to reduce the levels of methane in air flowing through a coal bed by as much as 75% (2).

The results obtained during this preliminary experimental phase were sufficiently encouraging that further experiments were conducted. The results from Phase III (3) showed that the small scale experiments carried out during Phase II could be scaled up to allow treatment of 10 Kg quantities of coal. The rates of methane oxidation in the larger model silos were as high as those observed in the small scale tests. No differences in gas composition were noted if gas samples were withdrawn from either the airspace above the coal or from within the coal pile. This showed that diffusion of methane or oxygen was not limiting the rate of methane oxidation for this size of silo.

The pH activity profile of the strain of organismm used was broadened from that used in the previous study. Significant activity was found at lower pH values, particularily in the range 5.0-6.5. The organisms were most active in the range 6.0-8.0.

The strain of organisms isolated in the previous study were more fully characterized, particularily their temperature and pH profiles. Optimum temperature for bacterial activity was shown to be 33°C. Activity dropped rapidly when the temperature was raised above the optimum but only dropped slowly when it was lowered. The organisms were totally inactive at temperatures below -20°C and above 40°C but recovered when temperature was adjusted to more favourable values.

Methods were adopted to allow the organisms to be used with high sulphur Cape Breton coals. Specifically, three methods were found to allow the organisms to be used with the Cape Breton coals. Firstly, the pH profile of the organisms was adapted to lower values. Secondly, methods were found to

immobilize the organisms so that they did not come in contact with the coal. Thirdly, if the inorganic sulphur was removed the resulting coal did not inactivate the organisms.

Based on the positive results obtained further scale up was attempted. Small scale (30 litre) airlift bioreactors were used to produce sufficient bacterial cells to treat 3 tonnes of coal in a model silo. The silo, constructed of steel, had a diameter of 1.3 m and was 3.3 m in height. Gas sampling ports were located at 30 cm intervals in the side of the silo. The top and bottom were sealed to prevent any air leakage during experimental runs.

Two runs were carried out using CBDC washplant coal and a third was run using a Western Canadian coal. During the experimental runs conditions were varied by alternately flushing the head space with air (to mimic natural ventilation expected in ships) and completely sealing the silo.

When the silo was sealed there was a rapid utilization of oxygen until the silo was completely anoxic within a period of 2-3 days. When the head space was ventilated a methane gradient was created within the silo. Carbon dioxide also rose rapidly after addition of the bacteria. Methane concentrations within the silo were considerably lower after addition of the bacteria.

Results were not as successful with the Western Canadian coal because it only emitted low levels of methane and it was very finely ground thus decreasing gas diffusion rates through the coal pile.

The results of this phase are described in a CANMET Report (4).

Based on the continuing positive results obtained from this

project, a further phase of experimental work was initiated. During this phase the methane oxidizing bacteria were successfully grown in a 1800 litre bioreactor. Growth conditions were not optimized but good production of cells was achieved. These cells were used for two large scale (80 tonne) tests in Sydney, Nova Scotia.

The first test used cells from only one production run in the bioreactor. The cells were not concentrated and whole culture broths were used. This test was partially successful in that after a period of 3-4 days 64% of the methane desorbing from the coal was oxidized.

Based on this test a second test was conducted. The amount of bacterial activity added to the coal was six times greater in the second test. Since larger quantities of bacterial cells were used for this test the bacterial cells were concentrated by centrifugation before they were shipped to Sydney. This test showed very little bacterial oxidation of the methane. The data gathered during this test suggested that, perhaps, some unknown inhibitor present on the coal during the second test was responsible for the failure of the test.

An additional test carried out using the small (3 tonne) silo showed that the bacteria were active on aged coal but that the activity was less that that previously observed.

Preliminary experiments showed that MIBC was inhibitory to the methane oxidizing bacteria at low concentrations (1000-2000 ppm). Other potential inhibitors had no effect on the methane oxidation rates.

The results from this phase of the project are described in detail in a CANMET Research Report (5).

It was decided to carry out further research on this

project. During this phase of the work, a new strain of methane oxidizing bacteria was isolated from Cape Breton coal. This strain was partially characterized and was produced in the 1800 litre bioreactor.

Unfortunately, this strain of methane oxidizing bacteria was not as stable as the original strain and lost some activity after harvest and on transportation to Sydney.

Barrel scale tests conducted in Sydney gave variable results but during the best tests greater than 90% of the methane was oxidized.

A rail car test was conducted in Sydney. There was an immediate drop in methane concentration in the experimental side of the rail car. However, this was followed by a steady rise in methane until the level in the experimental side was slightly higher than in the control side. There was a rapid drop in the oxygen concentrations in the experimental side and it was concluded that poor oxygen availabilty reduced the amount of methane which the bacteria could oxidize. After a period of several weeks the amount of methane oxidized approached 50%.

This was confirmed when it was pointed out by CBDC personnel that the fines content of the coal had increased from 7-24% in the period between the first and second tests. This increased fines content was, most likely, responsible for the reduced availability of oxygen within the coal pile.

The results of this phase of the project are described in a CANMET report (6).

Several methods to circumvent this problem were suggested. Firstly, a large scale test could be carried out using coal with a lower level of fines.

Secondly, the bacterial cells could be immobilized onto a porous support which would be placed on top of the coal.

Thirdly, oxygen could be provided to the bacteria in the form of hydrogen peroxide.

At first, it was thought that it would be impractical, for logistical reasons, to use coal with a low level of fines. Thus the emphasis was intially put on the use of immobilized cells. However, at a meeting in late November, 1989, it was decided to use low fine content coal rather than the immobilized cells. It was also decided to carry out some small scale tests before committing to a large scale test in Sydney.

The results of these experiments are described in this report.

EXPERIMENTAL METHODS

1 Coals Used in this Study

The coal used for all the experiments described in this report was a metallurgical coal from CBDC's Victoria Junction Coal Preparation Plant. The proximate analysis of this coal is shown in Table 1.

2 Strain of Methane Oxidizing Bacteria Used

Since the strain of bacterial methane oxidizing bacteria isolated from Cape Breton coal was so unstable it was decided to try and revive the initial strain which was first isolated from a Western Canadian coal mine.

A small aliquot of concentrated cells left over from the December 1987 large scale test had been stored in the fridge since that time. A small sample of concentrated cells were added to a 1 litre shake flask which contained 250 mL of Bushnell Haas liquid medium. The flask was sealed with a rubber membrane and 60 mL of methane added via a serum cap fitted to the side arm. The flask was shaken at 250 rpm for a number of days. Gas samples were withdrawn at daily intervals to check for bacterial oxidation.

After one week the cells were transferred onto fresh medium and further incubation in a methane atmosphere was continued. After several transfers an active culture was obtained. Five litres of bacterial broth was prepared in shake flasks and was then used to inoculate one of the 30 litre airlift bioreactors.

Bushnell Haas medium consists of the following ingredients:

Magnesium sulphate	0.2 g
Calcium chloride	0.02 g

TABLE 1

Coal Used in This Study

CBDC Washplant [1]

Rank	High volatile bituminous	
Ash content	3.0%	
Volatiles	36.0%	
Sulphur	1.4%	
Water content	8%	
Size distribution	+ 1 inch	2.5%
	+1/2 inch	13.1%
	+1/4 inch	17.9%
	+1/8 inch	16.6%
	+ 28 mesh	32.7%
	- 28 mesh	17.2%

[1] SGS Certificate analysis, A87-2498, September 1987

Monopotassium phosphate.....1.0 g
Dipotassium phosphate.....1.0 g
Ammonium nitrate.....1.0 g
Ferric chloride.....0.05 g
Tap water.....1000 mL

2 Bioreactor Used in this Study

The only bioreactor used for the experiments described in this report was the 30 litre airlift bioreactor used in previous phases of the project.

The 30 litre airlift bioreactor was constructed from ABS plastic tubing. The main compartment in the bioreactor was 10 cm in diameter and was 2 m high. An external draft tube of 5 cm diameter was connected via 90° elbows and two "T" junctions were fitted as shown in Figure 1. An additional piece of 12 cm tubing was attached above the top junction to prevent overflowing of the reactor when air was sparged through it. The total volume of the reactor was 30 litres but the nominal working volume was reduced to 25 litres due to gas holdup. Air was introduced to the reactor via a piece of tygon tubing which entered through the reactor top and was carried down inside the main compartment to a point approximately 15 cm above the bottom junction. A porous gas sparger was attached to the bottom of this tubing. A second tube exited from just inside the reactor and was vented outside the laboratory. Methane was bled into the air supply line and the flow rate was adjusted so that methane concentrations in the exiting gas were kept within the range 1-2% (v/v). This bioreactor was used in a fed batch mode with a 20% inoculum in a non-sterile manner.

3 Monitoring of Cell Growth

Growth and activity of the organisms were monitored daily by measuring the following parameters:

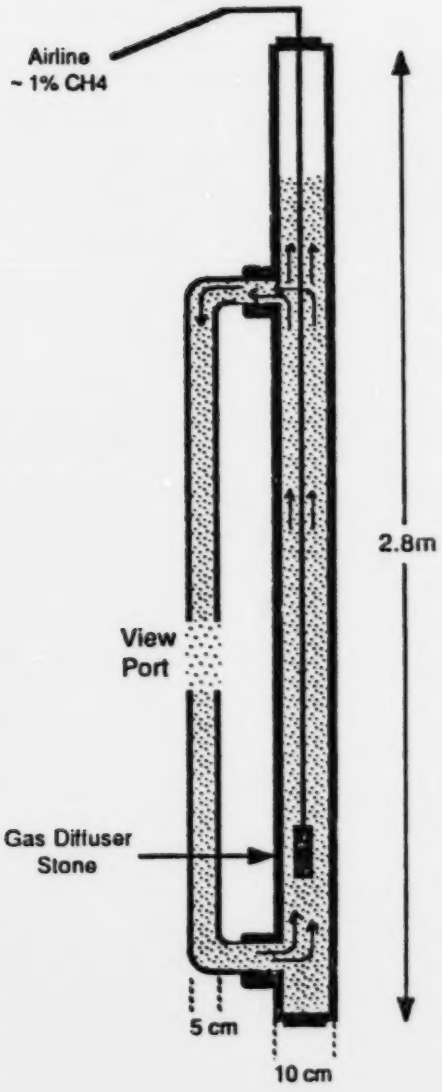

Figure 1. 30 Litre bioreactor used in this study

(i) methane oxidation activity (see section 4)
(ii) optical density of the culture broth at 600 nm
(iii) the purity of the culture was estimated by examing small aliquots of the broth under the microscope.

4 Methane Oxidation Measurement

The standard method for measuring methane oxidation rates described in a previous report (4) was used in this study. Briefly, five mL of a culture broth or cell suspension were added to a 20 mL serum vial. The vial was capped with a serum cap and 6 mL of methane added via a syringe. The initial methane concentration was measured by gas chromatography and the vial was incubated for 5 hours on a rotatory shaker (300 rpm) at 22°C. The residual methane concentration was measured and the difference between initial and final concentrations of methane was a direct measurement of the methane oxidizing activity of the samples. When highly concentrated cell suspensions were used the incubation period was reduced to 3 hours.

5 Small Scale Experiments Using Porous Coal

Samples of coal with a low fines content were shipped from CBDC's Victoria Junction Coal Preparation Plant. This coal was approximately 14 days old when it was received in Calgary.

Approximately 4 Kg of this coal was mixed with bacterial cells obtained from 1500 mL of culture broth. These cells were concentrated by centrifugation and were resuspended in 200 mL of fresh Bushnell Haas medium. The coal was placed in the test rig shown diagramatically in Figure 2. Air was blown across the top at a rate of 50 mL per minute. Samples were withdrawn from the sampling ports and from the exhaust air. These samples were anaylzed by gas chromatography for

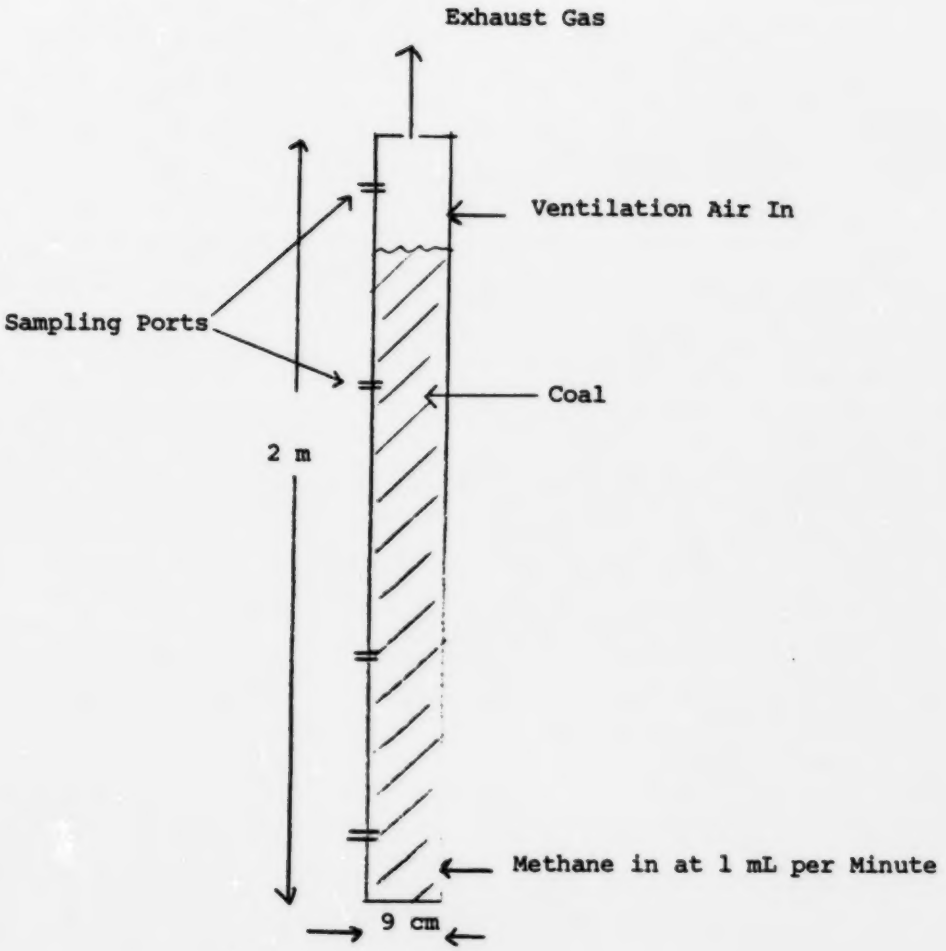

Figure 2. Test Rig Used for the Small Scale Experiments with Porous Coal

methane, carbon dioxide and oxygen.

A control rig which only had the Bushnell Haas medium was run at the same time to provide the control data.

After the experiment had been run for several days methane was introduced at the bottom of the coal pile to mimic the higher levels of methane which would be expected in a ship hold. The rate was 1 mL per minute which was calculated to give the same flux of methane through the surface layer as was found in the first of the large scale tests.

6 Small Scale Test Carried Out in Sydney

The concentrated cells obtained from approximately 15 litres of culture broth were shipped to Sydney, Nova Scotia. These cells were suspended in fresh Bushnell Haas medium and were added to 15 Kg of porous coal contained in a 5 gallon plastic bucket. A control experiment was also set up without the bacteria. Air was blown across the top of both containers to mimic natural ventilation. The exhaust gases were analzed for methane and carbon dioxide using an infra red technique.

7 Immobilized Cells

A preliminary experiment was carried out to test four different media. These included two fish filter materials, diatomaceous earth and a silica based catalyst support. Five mL of cell broth were added to each of the media in a 20 mL serum vial. The vial was capped and 6 mL of methane added. Initial methane concentrations were measured and the vials were incubated (no shaking) at 22° for 5 hours. The final methane concentration was measured and the methane oxidation activity was calculated as the five hour difference in methane concentration.

A large scale test was conducted in a ABS tube 7 cm in diameter and 1 m high. The cells from 1 litre of broth were concentrated by centrifugation and were sprayed on glass fibre insulation material before being placed in the tube. Sixty mL of methane were injected into the bottom of the apparatus and gas samples were withdrawn from the sampling points at 15 minute intervals. Unfortunately, only a few preliminary runs were carried out in this rig before the emphasis was switched to work with the porous coal.

8 Hydrogen Peroxide

This experiment was carried out as described in Section 4 except that various concentrations of hydrogen peroxide were added to the vials.

9 Long Lasting Liquid Foams

Two types of foam were tested, a natural protein foam (egg white) and a commercial chemical foam (fire supressing foam) from 3M. Small aliquots of concentrated cell suspension were aded to the foam and the foam was placed in a 20 mL serum vial. The vials were capped and 6 mL of methane added. After incubation at 22°C (non shaking) the amount of methane oxidized in a five hour period was measured.

10 Analytical Techniques

Necessary chemical and proximate analyses of the coal used in this project were carried out by a commercial laboratory following established ASTM methods or were provided by the coal companies.

Gases were analyzed by gas cromatography using inhouse facilites. The experimental details of this technique and other methods are outlined in the appendix.

RESULTS

1 Isolation of an Active Culture from the Old Strain

After approximately two weeks in a shake flask culture an active culture was obtained. After several transfers the strain was grown in the 30 litre airlift. The methane oxidation rate obtained with this new strain was only about 67% of that obtained with the previous two strains which had been used. In addition, there appeared to be difficulties with the production since the growth kinetics were not as reproducible as with the other strains. Some times the strain grew very well and active cultures were obtained in 4-5 days. Other times the culture grew very slowly and only low levels of activity were obtained. Time constraints prevented further work on obtaining a better strain and this strain was used for further work.

2 Characterization of the New Strain

This new strain appeared to be quite similar to its parent strain i.e. the strain originally isolated from the Western Canadian coal. However, there were a number of key differences. Firstly, as mentioned in the previous section, the maximum activity obtained in the bioreactor was only 67% of that of the parent strain. Secondly, the strain was not as stable as the parent strain and became discoloured after a few weeks, even when stored at refridgerator temperatures. These differences may explain some of the results obtained with this strain.

3 Addition of the Bacteria to Porous Coal

The results of two experiments are illustrated in Figures 3-8. As can be seen when no additional methane is added to the coal there is a very significant lowering of the methane throughout the experimental test. In addition, large

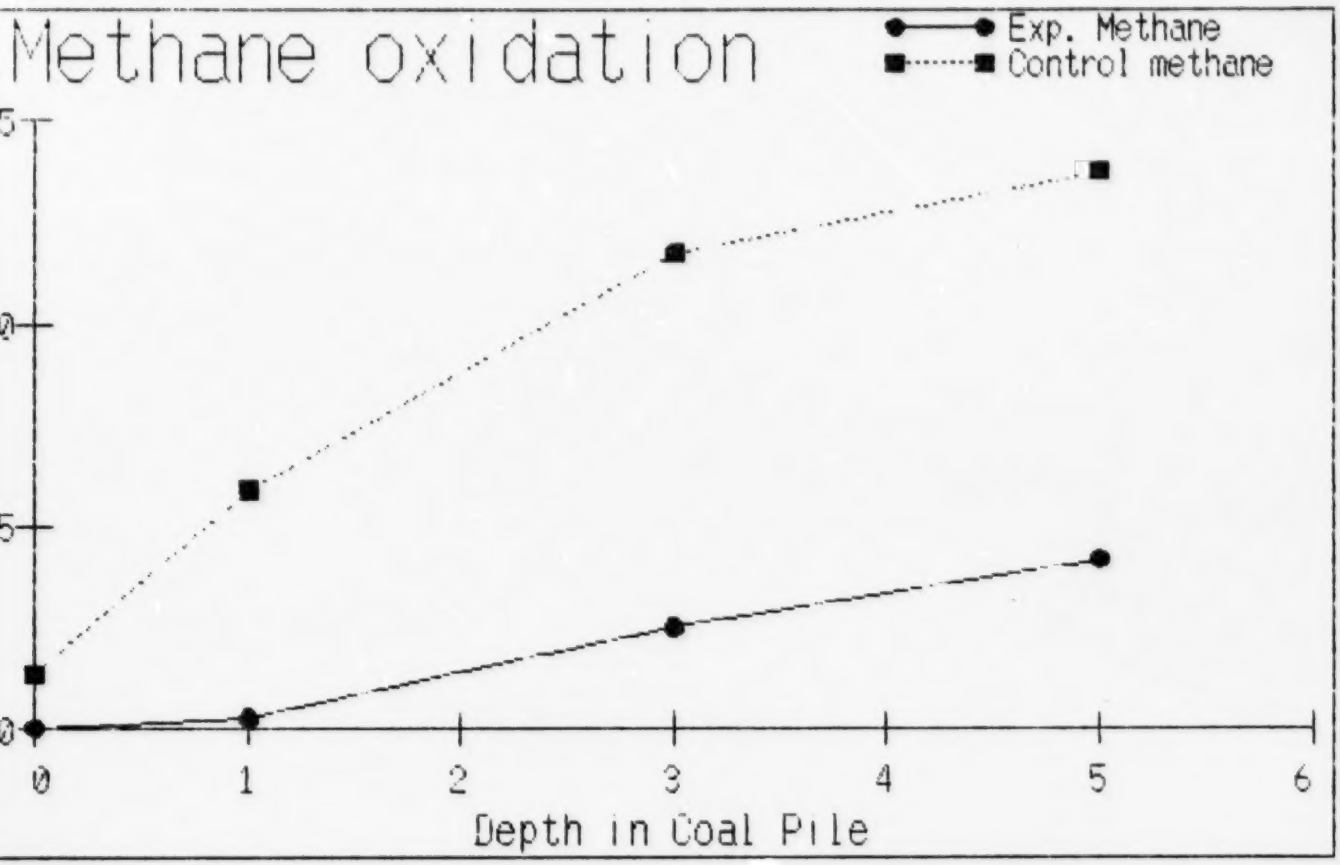

Figure 3. Small scale test with no additional methane

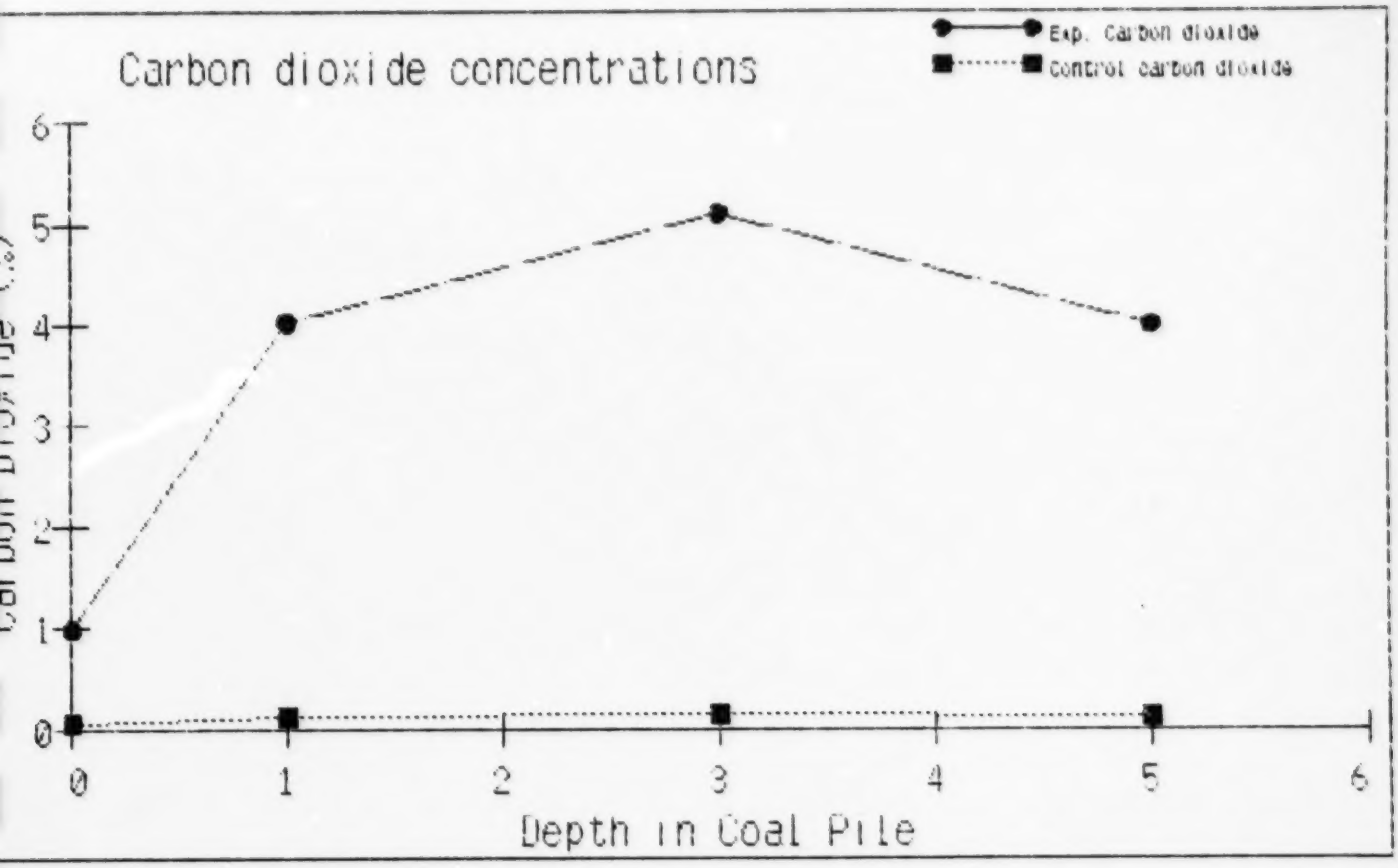

Figure 4. Small scale testwith no additional methane

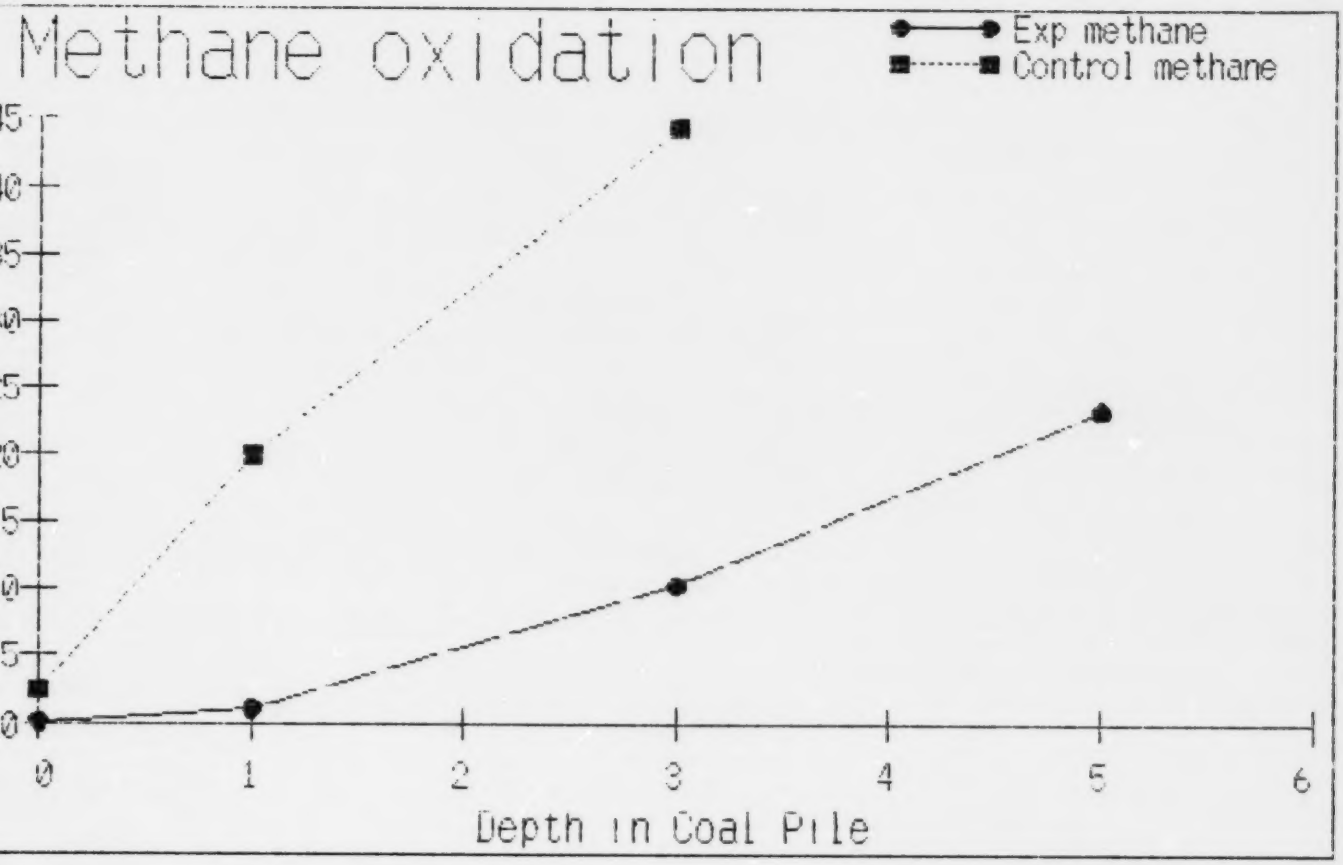

Figure 5. Small Scale test with methane at 1 mL/minute

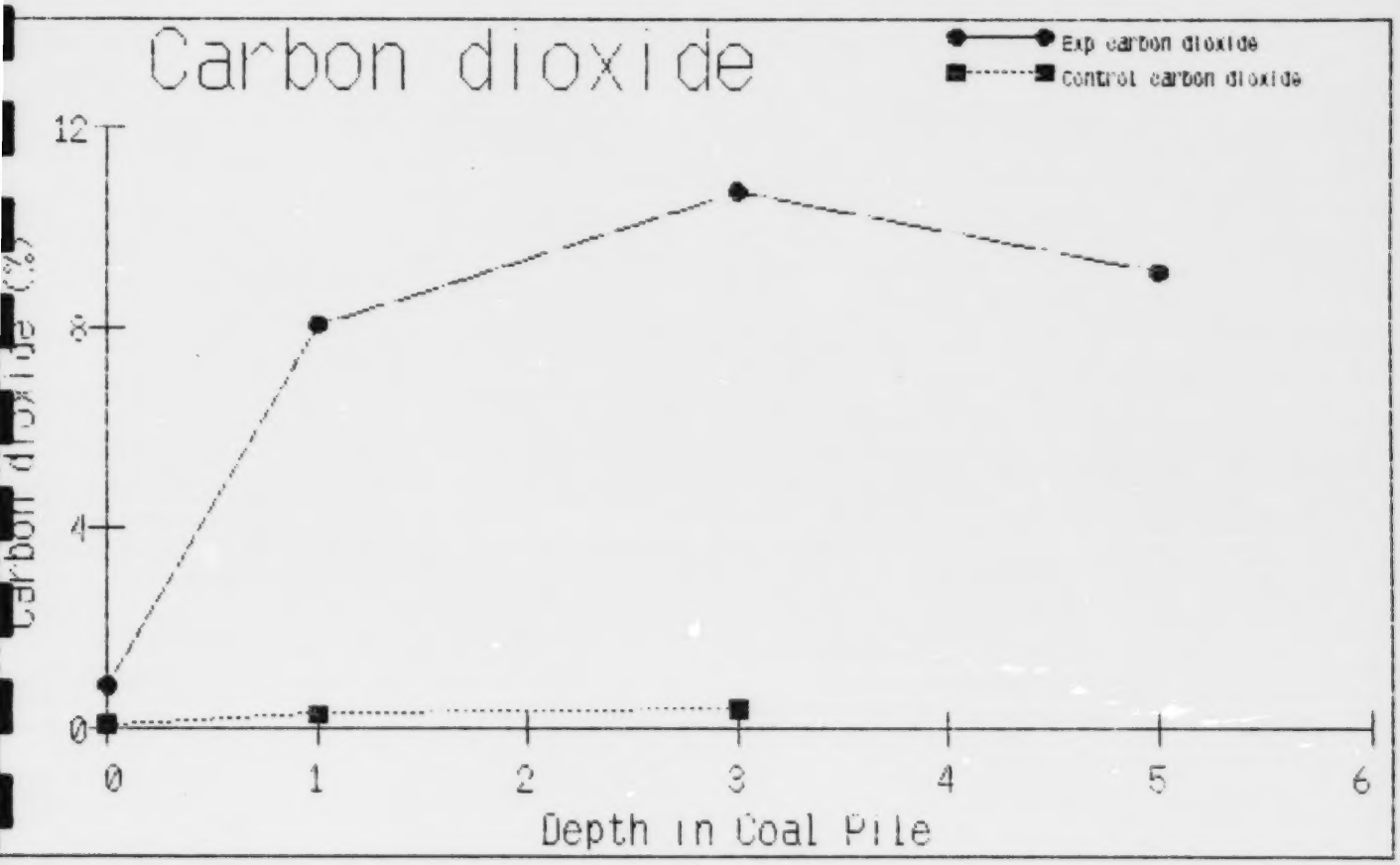

Figure 6. Small scale test with methane at 1 mL/minute

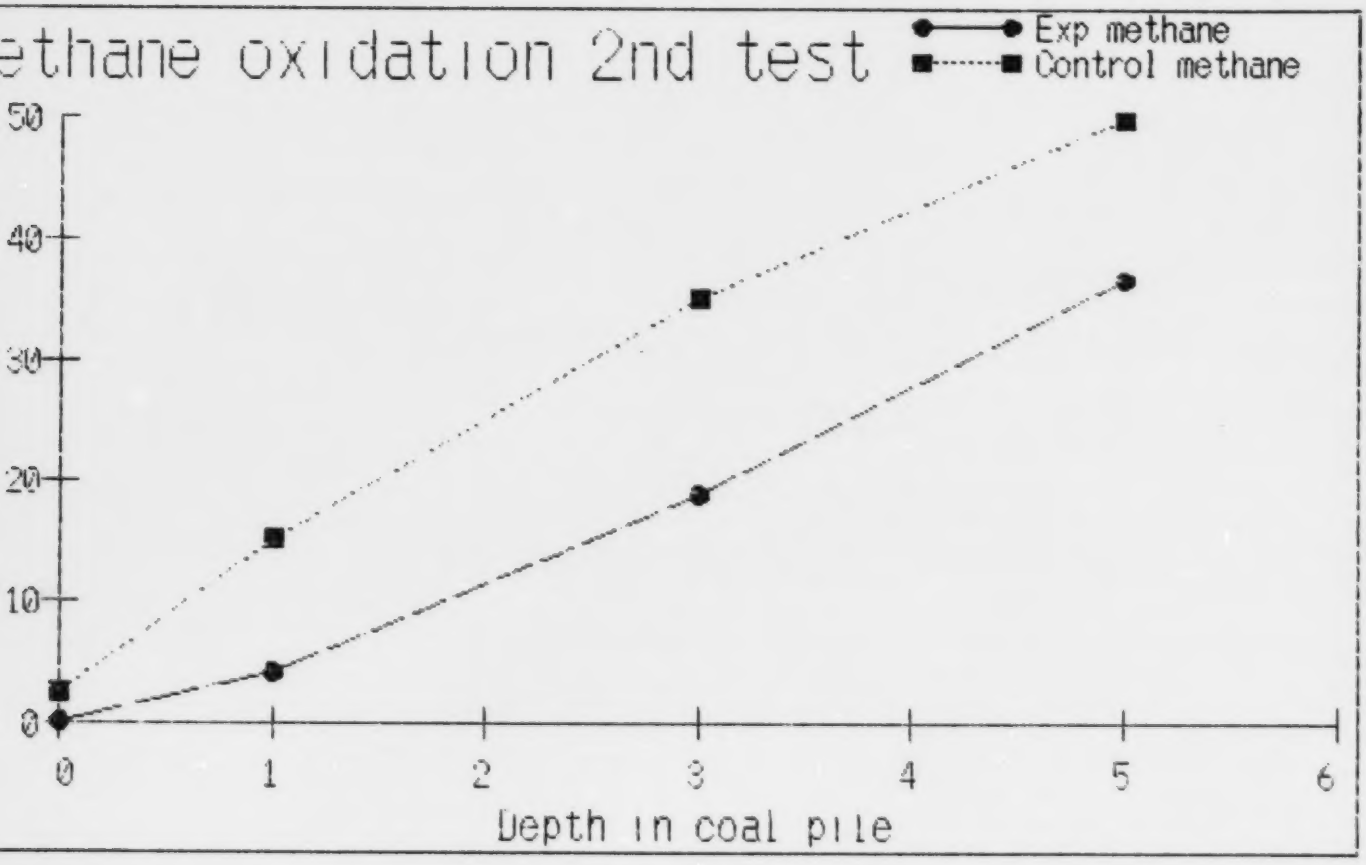

Figure 7. Second small scale test with methane at 1 mL/minute

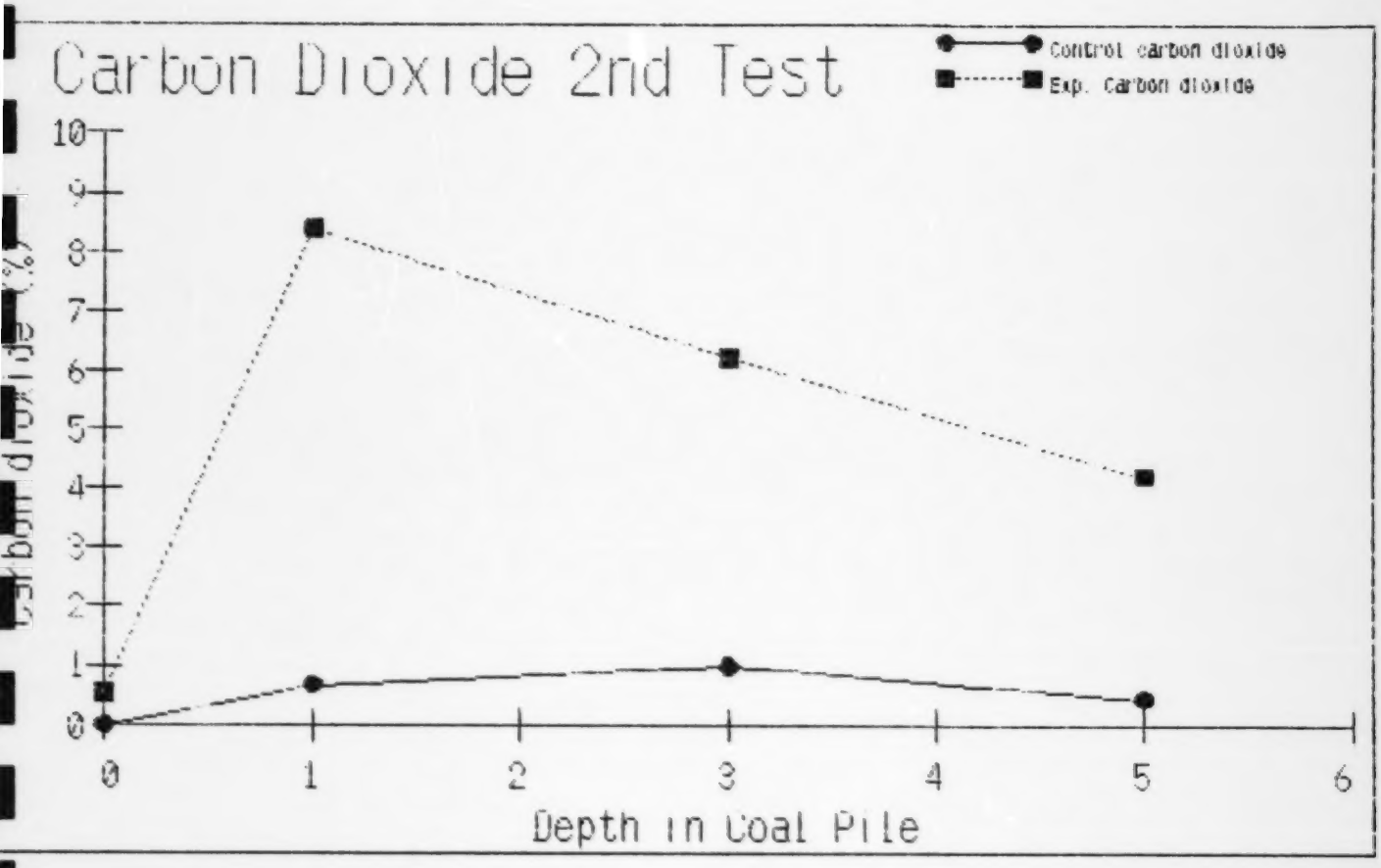

Figure 8. Second small scale test with methane at 1 mL/minute

quantities of carbon dioxide were produced, both in the exhaust gas and within the coal pile. When methane was added to the bottom of the coal pile at a rate of 1 mL/minute to mimic the use of deeper piles of fresh coal there was still significant reduction of methane in the exhast gas and within the coal pile. Measurement of oxygen showed that oxygen was detectable to a depth of 3 feet into the coal pile. This is illustrated in Table 2.

The results obtained from the second test were not as dramatic but still showed very good methane oxidation.

2 Small Scale Test Carried out in Sydney

The results of a small scale test carried out in Sydney by CBDC personnel are shown in Figure 9. As can be seen the results of this particular test were not encouraging in that only approximately 40% of the methane emitted from the coal was oxidized. This was significantly less oxidation than was obtained during the first of the large rail car experiments carried out in the summer of 1987.

3 Small Scale Experiments Using Hydrogen Peroxide

The results from this test are shown in Table 3. As can be seen this strain is extremely sensitive to the presence of hydrogen peroxide. Good methane oxidation rates could only be obtained at such low concentrations of hydrogen peroxide to make this approach to oxygen addition completely impractical.

4 Immobilization of the Bacteria in Liquid Foams

When the bacteria were immobilized in the protein foam they retained 100% of their activity. However, the protein foam was not stable and broke down over a period of 24 hours. The results using a commercial foaming agent were not successful

TABLE 2

Oxygen Concentrations Within the Coal Pile

	Control	Experimental
Exhaust Gas	18.7	15.46
One Foot	17.7	8.4
Three Feet	16.8	0.0

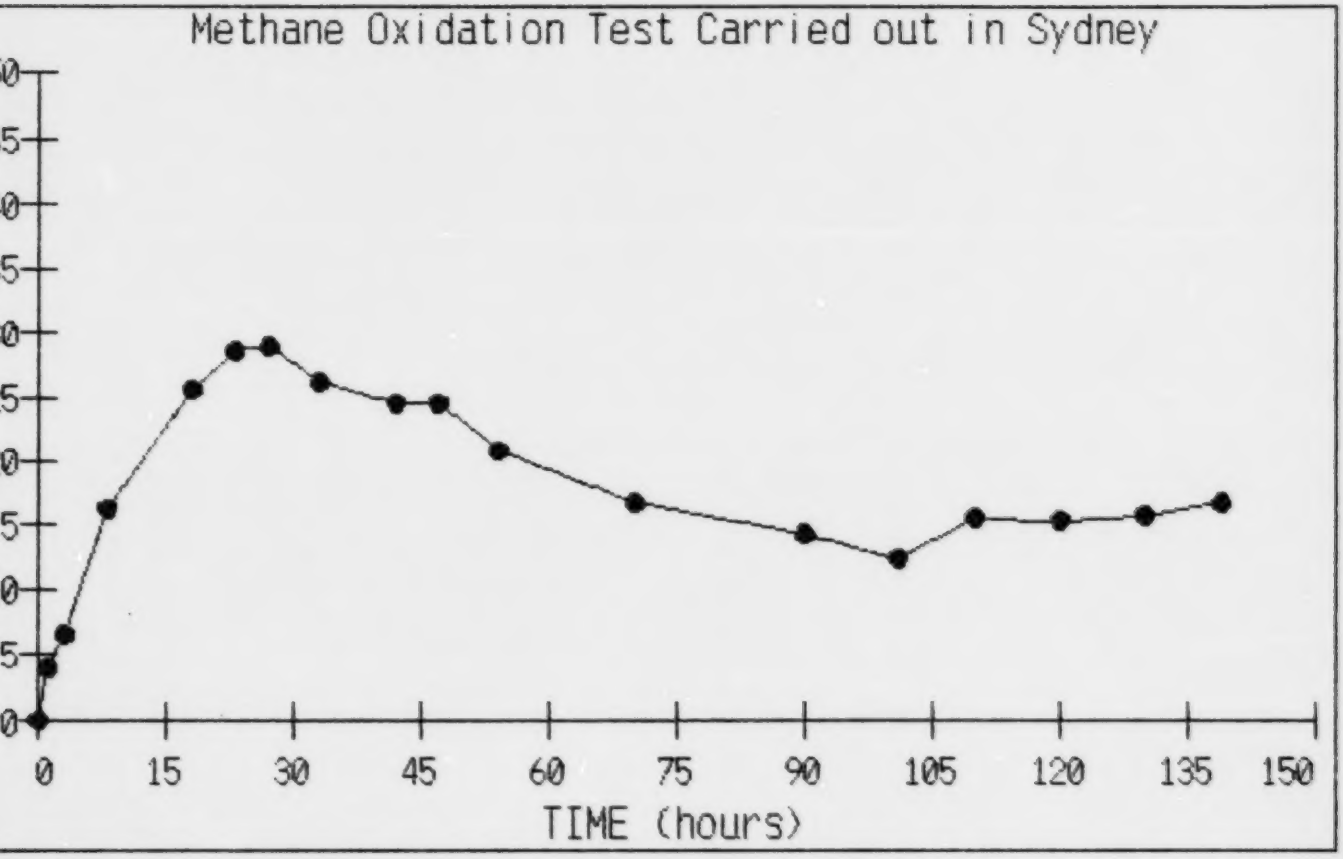

Figure 9. Small scale test carried out in Sydney

TABLE 3

Effects of Hydrogen Peroxide on the Bacteria

H_2O_2 Concentration	Relative Activity
0.0%	100.0
0.125%	28.1
0.25%	19.0

since this foam completely inhibited the bacteria. In addition, this foam broke down in a few hours. This foam could be stabilized using a chemical cross linking agent but this was not done because of the toxicity of the foaming agent. The new long lasting foams were not available to the company representatives who visited our laboratory and provided the samples of the commercial foam. This is an approach which should be studied in more detail in the future.

5 Immobilization of the Bacteria on Fibre Material

The results of preliminary testing of a number of different immobilizing supports are shown in Table 4. The fish type filter material was chosen for further testing on a larger scale. This material was not rigid enough when used on a larger scale. To overcome this lack of physical strength fibre glass insulation material was tested. This material gave superior physical performance and was used for the further tests.

The results of the experiments using cells immobilized on glass fibre material are shown in Table 5. As can be seen good methane oxidation rates were obtained in these preliminary experiments. Unfortunately, just as this system was being set up the emphasis for the project was changed from immobilized cells to the use of porous coal. Thus the results reported in this section are only of a preliminary nature. Further experiments should be conducted to verify the findings.

TABLE 4

Small Scale Experiments on Immobilization Supports

Type of Support	Findings
Fish Tank Filter	Good activity, good stability, poor physical properties
Diatomaceous Earth	Good activity initially, poor stability, good physical properties
Silica Based Industrial Catalyst Support	No activity, poor stability, excellent physical properties
Fibre Glass	Good activity, good stability good physical properties

TABLE 5

Large Scale Immobilized Cell Experiment

	Gas Concentrations (%)	
Time (Minutes)	Methane	Carbon Dioxide
0	0.0	0.12
15	0.14	0.24
30	0.27	0.27
90	0.38	0.57
105	0.32	0.71
120	0.28	0.85
150	0.18	1.07
Theoretical if no microbiological activity	1.5	0.03

DISCUSSION

The results from previous studies (4,5) showed that the lack of oxygen within the coal pile was limiting the bacterial oxidation during the large scale tests carried out in Sydney, Nova Scotia.

A further series of experiments was planned to see if ways could be devised to overcome this problem. In the previous report several methods were proposed. These included:

- o use porous coal similar to that used during the first large scale test
- o immobilize the bacteria in the air space above the coal
- o use hydrogen peroxide to provide additional oxygen to the bacteria

The results from this phase of the study are discussed below.

1 STRAIN OF BACTERIA USED

As described in the results section the strain of bacteria used in this phase was cultured from an old sample of the original Western strain. However, this substrain did not grow as well as the parent strain and was less active at its maximum activity. In addition, it was not as stable as the parent strain. Because of the limited time scale available for this project it was decided to proceed with this new strain rather than attempt to isolate a superior strain.

It is unfortunate, in retrospect, that sufficient funds were not available to continue to subculture the original strain to keep it in a viable state so that it could be used at a

later date.

2 IMMOBILIZED CELLS

The preliminary experiments showed that fibrous material is the best for use in immobilized cell work. Fish filter material was best on a small scale but was too soft for the larger scale work. Thus glass fibre insulation material was used. The preliminary larger scale experiments showed that this material could be successfully used. Further experiments should be carried out on methods to immobilize higher loadings of cells onto the immobilizing support. Also, experiments should be carried out to establish the linearity of cell loading versus activity at very high loadings. Experiments were carried out previously on the effects of concentrating the cells but only at moderate concentration factors (up to five times).

3 CELLS IMMOBILIZED IN LONG LASTING LIQUID FOAMS

The results from these experiments were not conclusive. However, the results were sufficently encouraging that further work in this area is justified. The protein foam showed that the bacteria were active when suspended in a foam form. If some method could be found to stabilize this type of foam (without inhibiting the bacteria) then this would be a most effective way to immobilize the bacteria. The ease of application of such a system makes it the only method of immobilization which would be practical for use in ship holds.

The other foam tested inhibited the bacteria and hence could not be used. Other foams should be tested to see if a foam exists which is both long lasting and does not inhibit the bacteria. A large number of potential foaming agents exist so there is a good chance that one with the correct properties can be found. Obviously, such a comprehensive

search was outside the scope of this phase of the project.

4 USE OF HYDROGEN PEROXIDE

The substrain used in this part of the work was inhibited at relatively low concentrations of hydrogen peroxide. It is not known whether insensitive strains of methane oxidizing bacteria exist which could be used to breakdown hydrogen peroxide to provide sufficient oxygen for use in the ship holds.

5 USE OF POROUS COAL

The results from the small scale tests carried out in Calgary confirm that porous coal allows for good access of oxygen to the bacteria. This test was set up to mimic the first large scale test. The methane flow rate (1 mL/minute) was chosen to approximate the flux of methane that occurred during that test. It is interesting to note that the level of carbon dioxide obtained in the recent test was very similar to that produced in the first large scale test, both in the exhaust gases and within the coal pile. Thus the small scale tests confirm that the first large scale test was successful due to the porous nature of the coal used. The results from the small scale test showed that a large scale test should be successful if the porous coal was used.

6 SMALL SCALE TEST CARRIED OUT IN SYDNEY

The results from this test were much poorer than expected from the results of the tests carried out in Calgary. No satisfactory explanation can be given except that as noted earlier the cells were not as stable as those used in the first large scale test and may have lost much of their activity during storage in Calgary, transport to Sydney and storage in Sydney. It should be noted that the percent methane oxidized was calculated from the amount of carbon

dioxide produced and assumed that 1 molecule of methane gives rise to 1 molecule of carbon dioxide after microbial oxidation. However, this assumption does not always hold since the ratio of carbon dioxide formed to methane oxidized is variable and is always much lower than expected, especially with fresh cells when a ratio of 0.5 has been noted in the past. Thus the actual amount of methane oxidized in this experiment could be substantially higher than the 30-40% reported.

The results of this particular test were in fact worse than several of the barrel scale tests carried out in Sydney last year in which the low porosity coal was used. In these test 80-90% of the methane was oxidized versus only 40% in the current test. Further experiments on this scale should have been carried out with fresh and active bacteria to confirm or negate the results. Lack of adequate financial resources prevented this from occurring.

CONCLUSIONS

1 The small scale tests carried out in Calgary using porous coal confirmed the results of the first large scale test. However, a small scale test carried out in Sydney did not confirm these results. During this test only 40% of the methane was oxidized. Why this should be so is unknown but the most likely explanation is that the cells had become inactivated before testing. The cells certainly did not look to be in good condition before shipment to Sydney. The authors of this report conclude that the use of porous coal would overcome the difficulties experienced with the last two large scale tests.

2 A small scale test carried out in Sydney was not successful. No reason can be given for the failure except to note that the bacterial cells were not stable and may have been inactivated before the test was conducted. In addition, the amount of methane oxidized was most likely underestimated.

3 Hydrogen peroxide is not an acceptable way to provide oxygen into the coal pile since it inactivates the methane oxidizing bacteria.

4 The methane oxidizing bacteria are active when suspended in a liquid foam. However, ways have to be developed to increase the longevity of the foam.

5 Preliminary experiments with the bacterial cells immobilized on glass fibres indicate that this would be an acceptable method to keep the bacteria separated from the coal. However, the potential for a temperature rise would have to be studied.

RECOMMENDATIONS FOR FURTHER RESEARCH

1 More research is required at a smaller scale. For example, no experiments have been carried out on the effects of the high concentration factors which are now being used. Experiments should be carried out both with the concentrated cells added to coal and when immobilized onto solid supports.

2 The stability of the two most recent strains is not satisfactory when the cells are produced in Calgary followed by shipment to Sydney. A more stable strain must be used.

3 The stability of the new strains when added to coal should be studied since inadequate data have been gathered to show how stable they are when applied to the coal.

4 Only once these questions have been answered in a satisfactory manner would it be prudent to continue with another large scale test. The technology works but more basic information on the system must be aquired to ensure that failures do not occur in the future.

5 Adequate resources must be given to a project of this nature if positive results are to be obtained. The use of living organisms dictates that the project must be viewed as one long continuous experiment rather than a series of discrete phases which can be turned off and on at will. Too much time has been spent in the past in returning to the previous high point with regards to the activity of the strains rather than continuing from that point.

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APPENDIX

Gases were analyzed using a Perkin Elmer Sigma 3B gas chromatograph using the following conditions:

Column	Supelco carbosieve SII 10' x 1/8" s.s.
Oven temperature	75°C for oxygen, 225°C for methane and carbon dioxide
Injector temperature	250°C
Detector temperature	250°C
Carrier gas	helium at 30 mL/min.
Detector type	hot wire
Sample loop	0.01 mL

pH measurement was made using a combination electrode and a Fisher Accumet pH meter (model 425 digital).